

A comparison of the inactivity of ozone as a disinfectant in the dry state with its action in the presence of water suggests a superficial resemblance with other gases, such as chlorine and sulphur dioxide. In the absence of further experiment, however, it would not be possible to press the analogy too closely.

In the dry state, and under the conditions in which it occurs in nature, ozone, then, is not capable of any injurious action on bacteria so far as can be judged from our experiments; and we conclude that any purifying action which ozone may have in the economy of nature is due to the direct chemical oxidation of putrescible organic matter, and that it does not in any way hinder the action of bacteria, which latter are, indeed, in their own way, working towards the same end as the ozone itself in resolving dead organic matter to simpler non-putrescible substances.

“On the Functions of the Bile as a Solvent.” By BENJAMIN MOORE and WILLIAM H. PARKER. Communicated by Professor SCHÄFER, F.R.S. Received January 24,—Read February 14, 1901.

The purpose of the biliary secretion and the uses of that fluid in digestion and otherwise have furnished much material for discussion to the physiological chemist, and the discussion has given rise to many ingenious but widely different theories.

The bile, unlike all the other digestive fluids which are secreted into the alimentary canal, has no specific action upon any of the three classes of food-stuffs. It contains small amounts of cholestearin and lecithin, and of other substances which are obviously to be regarded as excretory in character. It is necessary in the intestine for the complete absorption of the fats in normal amount, but even in its absence a considerable amount of fat can still be absorbed. The constituents which it contains in solution in largest quantity are the sodium salts of certain acids called the bile acids, and these bile salts are not excreted, but are reabsorbed, and undergo a circulation in the blood known as the circulation of the bile.

These few statements briefly summarise our experimental knowledge as to the action and physiological properties of the bile, and have given a basis to many theories.

It has been argued by some from the fact that bile contains no digestive enzyme, and from the presence in the fluid of certain constituents which are certainly excretory, that the bile is to be regarded purely as an excretion; but this view gives no explanation of the re-absorption of the bile salts, which are the most abundant constituent.

By others the bile has been regarded as an anti-putrefactive, although it readily undergoes putrefaction itself. Others, without much experimental proof, have suggested that it stimulates the intestinal epithelium and increases peristalsis, but even if this be allowed it leaves much of the action of the bile untouched. While it is universally admitted that bile exhibits at most only unimportant traces of a digestive action on food-stuffs, some observers state that its presence favours and increases the activity of other digestive fluids upon carbohydrates, fats, or proteids, and see in this an important function of the bile.* On the other hand, it is stated by other experimenters that this aiding power of the added bile is no more than can be explained by the alteration in chemical reaction of the mixed fluid.†

With regard to the action of bile in favouring fat absorption, one view which has been held is that the bile alters the physical character of the intestinal epithelium when it wets it, and in some physical way makes the conditions more favourable for the taking up of emulsified fats. Since it is very probable, however, that all the fat is absorbed in some soluble form, and not as an emulsion, this theory of biliary activity falls to the ground.

It was first suggested by Altmann,‡ mainly from histological observations, that bile aided fat absorption by dissolving the fatty acids set free from the neutral fats in the intestine. Marcet§ had shown before this that bile dissolves free fatty acids to a clear solution, and later Moore and Rockwood|| determined the solubilities of fatty acids in bile, and further demonstrated that in some classes of animals a certain amount of the fat was absorbed as dissolved free fatty acid.

The latter authors, while admitting that a considerable amount of absorption of fat as dissolved free fatty acid occurs in carnivora, and insisting upon the importance of bile as a solvent in this connection, showed from a consideration of the reaction of the intestinal contents during active fat absorption that in other species of animals practically all the fat was absorbed as dissolved soaps. Even in carnivora it was further shown that in addition to the absorption as free fatty acid dissolved by the bile, a considerable amount of absorption as dissolved soaps takes place.

The soaps formed in the intestine during the digestion of fat are chiefly sodium soaps. Now it has universally been taken for granted that these are easily soluble in water, and no one has considered any action of the bile as necessary to their solution in the intestinal con-

* Rachford, 'Journ. of Physiology,' 1899, vol. 25, p. 165.

† Chittenden and Albro, 'Amer. Journ. of Physiol.,' 1898, vol. 1, p. 307.

‡ 'Arch. f. Anat. u. Physiol.,' 1889, Anat. Abth. Supp. Bd., p. 86.

§ 'Roy. Soc. Proc. Lond.,' vol. 9, 1868, p. 306.

|| 'Roy. Soc. Proc.,' vol. 60, 1897, p. 438; 'Journ. of Physiol.,' vol. 21, 1897, p. 58. (In this paper the literature of the subject is given.)

tents. But the process of preparing the sodium soaps easily demonstrates that *the mixed sodium soaps prepared either from beef or mutton suet are only very sparingly soluble in water*. When the mixture obtained by boiling the fat is thrown into cold water, practically none dissolves, and the excess of alkali can easily be washed off in this way. An increase in the amount of oleate present raises the solubility in water, so that a mixture of soaps obtained from pig's fat cannot be separated in this way. When the mixed soaps derived from beef or mutton fat are boiled with water, they do dissolve to a greater extent; but the solution sets, on cooling, to a stiff jelly, even when it contains as little as 2 per cent. of the mixed soaps.

It occurred to us, therefore, that it would be desirable to make comparative quantitative experiments as to the solubilities *at body temperature* of such soaps in water and in bile respectively, in order to determine whether bile possessed any function as a solvent in soap absorption from the intestine. Opportunity was also taken to prepare and test the solubility quantitatively of the so-called "insoluble soaps" of calcium and magnesium, as well as of the separated and purified oleates, palmitates, and stearates of sodium, calcium, and magnesium.

Attention has previously been given to the solubility of the magnesium and calcium soaps, so far as we are aware, only in a qualitative fashion; and the unqualified statement has in consequence been made by Neumeister* that these soaps are dissolved in the intestine by the agency of the bile.

There is, in addition to the solvent action of bile upon the various fatty derivatives in the intestine, another point of view from which we may regard the bile as a solvent, and ascribe to it a very important function connected with the excretion into the intestine from the liver of substances insoluble in water. It is well known that the bile contains cholestearin and lecithin, and although these bodies are not present in large percentage, they occur in greater quantity in the bile than in any other fluid in the body, and further this is the only channel by which these important degradation-products of metabolism are removed from the body.

Although the presence of these substances in the bile has long been known, no one, so far as we are aware, has drawn any inferences as to why they are excreted by the bile rather than any other excretory channel, nor recognised the importance of the change in the physical properties of the bile, whereby it is adapted for carrying off these waste products to the intestine, and so acquires a specific function possessed by no other fluid in the body.

Both lecithin and cholestearin are insoluble in water, and hence cannot be thrown out of the body in simple aqueous solution. This fundamental fact suggests inquiries as to how these substances are

* 'Lehrbuch der physiologischen Chemie,' Jena, 1897, p. 221.

carried in solution to the liver cells to be there excreted, as to how they are preserved in solution in the bile, and as to the extent to which each of them is soluble in that fluid.

Experiments were accordingly arranged to test the powers of the bile salts as a solvent for these two substances, which taken in conjunction with the known facts as to the reabsorption and circulation in the blood of the bile salts cast a considerable light upon the questions above outlined, and furnish a rational explanation of the so-called "circulation of the bile."

It is, in our opinion, in this property of acting as a solvent for substances which are insoluble in water, that bile has its main if not its only function, both in excretion and absorption.

Any other properties which have been ascribed to the bile are of very minor importance compared to this one. It enables us in the first place to explain clearly the part played by bile in fat absorption, for our experiments show not only that the solubilities of the soaps are considerably increased, but, which is of more importance still, that they are dissolved by the bile in a different physical condition from that in which they are held in solution by water alone, as is shown by the altered physical properties of the solution. Further, free fatty acid could not be held in solution in the intestine in the absence of bile. Again, it is impossible to see how such substances as cholestearin and lecithin could be excreted in the absence of some vehicle conferring solubility upon them.

Experimental Methods.

The bile salts used in our experiments were prepared by a usual modification of Plattner's method from ox bile. The bile was concentrated to a syrup on a water-bath, mixed into a paste with animal charcoal, extracted with absolute alcohol, filtered, and ether added to commencing precipitation. On standing, the bile salts were obtained in crystalline spherules, and these were purified by dissolving in alcohol and reprecipitating with ether.

The mixed sodium soaps employed were obtained by saponifying beef suet. Much labour was expended on various attempts to prepare these in a pure form; such as obtaining the free fatty acids in ethereal solution and neutralising with alcoholic potash, or extracting the soaps with hot alcohol in a Soxhlet apparatus and cooling out from the alcohol. These methods have practical difficulties, however, on account of the varying solubilities of the constituent salts in the organic solvents. Accordingly, a simpler method was found to yield better results. The fat was first saponified by slight excess of caustic soda, and the mixture of soaps thrown into a large excess of cold water,*

* Saturated solution of sodium chloride was at first used, but it was found that the mixed sodium soaps were so insoluble in *cold* water that no such saline

which dissolves out the surplus of alkali and inorganic salts. The soaps were next converted into free fatty acids by treatment with dilute hydrochloric acid, and the mixture of fatty acids was thoroughly washed by warming with water. The free acids were again converted into soaps by very slight excess of caustic soda, dissolved in boiling water, precipitated by cooling, washed with cold water, dried in a water bath, powdered, and kept in a glass-stoppered bottle.

The mixed calcium and magnesium soaps were prepared from these by precipitation from solution in hot water with calcium chloride and magnesium sulphate respectively, washing thoroughly with water, and drying on a water bath.

The pure oleic acid and oleates used were prepared from a sample of pure oleic acid by Merck.

The pure palmitic acid was obtained from bereberry tallow by repeated partial recrystallisation from alcohol until a constant and accurate melting point was obtained. The sodium soap was obtained by neutralising with caustic soda and recrystallising from hot alcohol; the magnesium and calcium soaps by precipitation of the sodium salt in hot aqueous solution by the appropriate salts, washing by decantation with cold water, and drying.

The pure stearic acid and stearates were similarly prepared from commercial stearin, and their purity tested by melting-point determinations for the free acid.

The lecithin used was prepared from yolk of egg by the following modification of the method of Hoppe-Seyler: The yolks were separated, beaten up into a common mass and extracted with five times their volume of 95 per cent. alcohol at a temperature of 50° to 60° C. for about two hours. The precipitated proteid and membrane was separated off by pressing through cheese cloth, the filtrate was allowed to cool to about 30° C. and separated from a certain amount of fatty oils which became pressed through along with the alcoholic extract. The alcoholic extract was evaporated down to a syrup at a temperature of about 60° C. on the water-bath, and then taken up in a small volume of absolute alcohol at a temperature of 40° to 50° C. This extract was next surrounded by a freezing mixture and kept at a temperature of -5° to -10° C. for some hours, which precipitates the greater part of the lecithin. This was removed by decantation and filtering through a chilled funnel, purified by again dissolving in

precipitant is required. Not even any sodium oleate is dissolved by the cold water, as can be shown by first throwing into cold water, then removing the soap and saturating the water with sodium chloride, when scarcely a trace of a precipitate is obtained. Nor are acid soaps formed by this method of preparation, on account of dissociation of the alkali, for on incineration of the soaps and titration of the residue as sodium carbonate, we have obtained almost the theoretical yields required for neutral soaps.

a small volume of absolute alcohol, and once more cooling out of solution. The final product was dried in a desiccator over sulphuric acid for some days.

In the case of cholestearin the figures obtained for the solubility were so low, that pure cholestearin preparations were made from several sources in order to make certain of the result; but all the specimens gave a like result.

The cholestearin first used was prepared from a laboratory specimen by repeatedly recrystallising from ether and from hot alcohol. The second specimen was obtained by repeated recrystallisation from hot alcohol and ether of the residue after taking out the lecithin from the hot alcoholic extract of egg yolk by means of a small volume of absolute alcohol as above described. Large characteristic cholestearin crystals were easily obtained by this method in great abundance. A third specimen was similarly prepared from ox brain, and a fourth from human gallstones by the usual method of extraction.

Comparative determinations were made of the solubilities in distilled water, in 5 per cent. aqueous solution of bile salts, in 5 per cent. aqueous solution of bile salts *plus* 1 per cent. of lecithin, and occasionally in ox bile. Two methods were employed in carrying out the determinations, which were all made at a temperature as close to that of the human body as possible, viz., at 37° to 39° C.

In one method, an excess of the substance of which the solubility was to be determined was heated to a temperature of 50° to 60° C. with the solvent; the mixture was allowed to cool to the required temperature, and then filtered through paper in a funnel kept at body temperature by a warm jacket. It was afterwards tested that the filtrate became clear, when it was once more heated to body temperature.

The percentage dissolved is then estimated by determining the amount of dissolved substance in a given volume, say 5 c.c., of the filtered solution. This is done by evaporating to dryness, extracting the fatty acids with ether (in the case of the soaps, after first converting into free fatty acids by the action of a mineral acid), and weighing after evaporating off the solvent.

This method has some practical disadvantages which have precluded its use except in the case of the determination of the solubility of the sodium soaps in bile. In the first place, a considerable amount of both solvent and solute must be used in order to obtain a workable quantity of filtrate. It is also difficult to filter with some of the substances tested, and on extraction of the evaporated solution with ether it is often impossible to obtain a clear ethereal solution. This method has therefore only been carried out in the case of the sodium soaps and bile. Here it has been used to determine the *maximum* amount which can be taken up by the bile from such a naturally-occurring mixture of

soaps as is obtained in the saponification of beef fat. When such a mixture is submitted to the solvent action of the bile it is found that more sodium oleate than palmitate or stearate is taken up, as is shown in the considerable reduction which is obtained in the melting point of the mixture of fatty acids dissolved and re-obtained from the bile as compared with the melting point of the fatty acids obtained from the mixed soaps before being acted upon by the bile. In fact, it is only when sodium oleate is also present that sodium palmitate and stearate are taken up by the bile in appreciable quantity. As a result of this, the figures obtained by this method, in the case of the mixed sodium soaps, must only be taken as indicating the maximum amount of soaps which the bile is capable of taking up from such a mixture at body temperature, and it must be remembered that the portion taken up has not the same composition as the mixture extracted, and that the solubility of the residue gradually decreases as the percentage of palmitate and stearate in it increase.*

The second method, which has chiefly been used in making the determinations, is to add the substance to be dissolved in small weighed portions at a time to a measured volume of the solvent contained in a test-tube and kept at body temperature by being immersed in a water bath provided with a thermostat. The mixture is stirred from time to time with a glass rod, and the substance to be dissolved is rubbed up with the solvent to hasten the process of solution. The amount added when solution ceases to be complete is noted, and from this a close approximation can be made to the percentage solubility. The approximation is the closer the smaller the amount of substance added each time, and the larger the volume of solvent which is taken. By using 10 c.c. of solvent and adding the substance in portions of 0.01 gramme at a time, it is thus possible to determine the solubility within one-tenth of a per cent. The method is somewhat laborious in making a first determination from the number of weighings, but in later determinations with the same solvent and solute it can be shortened by adding at once nearly the total quantity which it is known will be dissolved. Reliable results are obtained by this method in the case of determining the solubility of pure substances, but in a mixture of the soaps it gives a lower result than the total amount which the solvent will take up from the mixture, because the signal for stopping is here that point at which the maximum amount of the least soluble constituent of the mixture has been taken up. Thus a slight residue is obtained when even as little as 0.5 per cent. of mixed sodium soaps is added to bile at body temperature, and a somewhat heavier residue when water is

* A similar result is seen when the mixed fatty acids or soaps obtained by saponifying any naturally occurring fat are treated with a solvent in which they are not exceedingly soluble, such as hot alcohol, a residue of insoluble stearic acid or stearate is finally obtained.

employed as the solvent ; the amount of undissolved residue increases as the amount of mixed soaps added is increased, but it is obvious to the eye that a considerable amount of the later additions of soap are being dissolved, and, further, a determination of the melting point of the mixed fatty acids obtainable from the undissolved residue proves that this consists chiefly of palmitates and stearates.

This is interesting from the physiological point of view, since a similar separation must take place in the intestine, and the oleates be absorbed more readily and more rapidly than the palmitates and stearates.

RESULTS.

1. FREE FATTY ACIDS.—The mixed free fatty acids obtainable from beef suet are practically insoluble in distilled water at body temperature. When as little as 0.1 per cent. is added, the greater part remains undissolved in the form of melted globules ; but, on cooling down, a faint opalescence in the fluid indicates a slight degree of solubility. A 5 per cent. solution of bile-salts dissolves 0.5 per cent. of the mixed acids, and a 5 per cent. solution of bile-salts *plus* 1 per cent. of lecithin dissolves 0.7 per cent. The effect of the lecithin in increasing the solubility is clearly seen by heating simultaneously in two test-tubes, one containing bile-salts alone, and the other bile-salts *plus* lecithin, 0.5 per cent. of the fatty acids. The tube containing the lecithin clears first, and on cooling the two tubes a heavy precipitate is obtained in the case of the bile-salts only, and scarcely any precipitate in the solution containing lecithin in addition.

Oleic acid has the following solubilities :—Distilled water less than 0.1 per cent. ; bile-salt solution, 0.5 per cent. ; bile-salt *plus* lecithin solution, 4 per cent.*

Palmitic acid, in distilled water, less than 0.1 per cent. ; in bile-salt solution, 0.1 per cent. ; in bile-salt *plus* lecithin solution, 0.6 per cent.

Stearic acid, in distilled water, less than 0.1 per cent. ; in bile-salt solution, less than 0.1 per cent. ; in bile-salt *plus* lecithin solution, 0.2 per cent.

2. SODIUM SOAPS.—The mixed sodium soaps of beef suet, tested by the supersaturation method, yield to distilled water 2.23 per cent., and to ox bile (sp. gr. 1027) 3.69 per cent. The solubilities in the other solvents of the mixed soaps was not determined, because the constituents, for the reasons assigned above, are not taken up in proportionate quantities, and hence the figures have little value as quantitative results.

The above figures consequently give merely the maximum uptake of

* The bile-salt solutions employed invariably contained 5 per cent. of the mixed bile-salts of ox bile, and the bile salt *plus* lecithin solutions 1 per cent. of lecithin in addition.

soaps by bile from such a naturally occurring mixture, and do not mean that a mixture of soaps of unaltered composition is taken up to the extent indicated.

Of much more importance physiologically than the increase in *amount* of soap taken up, due to the presence of the bile salts, is the obvious physical change in character of the solution. After filtration in each case from the excess of undissolved soap, a difference is observable even at body temperature between the two solutions. The solution of slightly over 2 per cent. of soaps in distilled water is opalescent like a starch or dilute glycogen solution, while that of over 3 per cent. of the same soaps in bile is limpid and clear. On allowing the two solutions to cool to the temperature of the room, the physical differences become much more marked, for the more dilute distilled water solution sets into a stiff jelly so that the containing flask can be turned upside down without causing any alteration in the shape of the jelly, while the solution in bile remains quite limpid, and only a small part of the dissolved soaps passes out of solution as a *finely granular precipitate*. The formation of a jelly on cooling, in the case of the distilled water solution only, is not due to the fact that a larger quantity of soaps passes out of solution here on cooling; for no matter at what temperature higher than that of the body bile be saturated with the mixture of soaps, and hence no matter how much soap passes out of solution on cooling, it never forms a jelly, but always a precipitate and a clear supernatant fluid.

Now the formation of a viscid solution and ultimately of a jelly is one of the general properties of colloidal solutions, and hence the above-described experimental difference in behaviour probably indicates that soaps in solution in distilled water are in a more colloidal condition, and accordingly in a less diffusible and absorbable condition, than when dissolved in the presence of bile-salts.

Sodium oleate has the following solubilities—in distilled water, 5.0 per cent.; in bile-salt solution, 7.6 per cent.; in bile-salt *plus* lecithin solution, 11.6 per cent.

Sodium palmitate, in distilled water, 0.2 per cent.; in bile-salt solution, 1.0 per cent.; in bile-salt *plus* lecithin solution, 2.4 per cent.

Sodium stearate, in distilled water, 0.1 per cent.; in bile-salt solution, 0.2 per cent.; in bile-salt *plus* lecithin, 0.7 per cent.

3. CALCIUM AND MAGNESIUM SOAPS.—The usual statement that the “insoluble soaps” of calcium and magnesium are soluble in bile receives considerable modification when tested quantitatively, for the experiment shows that these soaps are only very sparingly soluble in bile. Neither the mixed calcium or magnesium soaps derived from beef suet nor their constituent salts, viz., the respective oleates, palmitates, or stearates, are at all soluble in distilled water, that is to say, the solubility in each case lies much below 0.1 per cent., which we

have taken as the lowest practicable limit in making our determinations. The solubility of the mixed calcium or magnesium soaps in bile is difficult to accurately determine on account of the undissolved residue of palmitate and stearate left behind. When even as little as 0.1 per cent. of either mixture is added to ox bile a residue is obtained. The magnesium soaps are somewhat more soluble than the calcium soaps, but in both cases the solubility is very low. In the case of the mixed calcium soaps, apparently none is taken up into the solution after 0.2 per cent. has been added; and in the case of the mixed magnesium soaps the same result is attained after the addition of about 0.4 per cent. Similar results are obtained in the case of the mixed soaps with bile-salt solution alone, and with bile-salt *plus* lecithin. A bile-salt solution (5 per cent.) ceases to dissolve more when 0.1 per cent. of mixed calcium soaps has been added or 0.2 per cent. of mixed magnesium soaps; and the figures are almost doubled when 1 per cent. of lecithin is dissolved in addition in the bile-salt solution used.

When the solubilities of the separated soaps in bile-salt, or in bile-salt *plus* lecithin, solutions are tested, it is found that the solubilities are only considerable in the case of the oleates; and here again it is seen that the magnesium salts are more soluble than the calcium salts.

Calcium oleate, in bile-salt solution, 0.2 per cent.; in bile-salt *plus* lecithin solution, 1.4 per cent.

Calcium palmitate, in bile-salt solution, less than 0.1 per cent.; in bile-salt *plus* lecithin solution, 0.9 per cent.

Calcium stearate, in bile-salt solution, less than 0.1 per cent.; in bile-salt *plus* lecithin solution, 0.4 per cent.

Magnesium oleate, in bile-salt solution, 3.2 per cent.; in bile-salt *plus* lecithin, 8.2 per cent.

Magnesium palmitate, in bile-salt solution, 0.2 per cent.; in bile-salt *plus* lecithin, 1.2 per cent.

Magnesium stearate, in bile-salt solution, less than 0.1 per cent.; in bile-salt *plus* lecithin solution, 1.0 per cent.

The physiological importance of the solubilities of the calcium and magnesium soaps in bile has, in our opinion, been much overrated. Although the figures above given show that the solubilities of the mixed soaps of calcium or magnesium are very low, and hence that the usual statement that these bodies are soluble must be modified, a point of more physiological import is that the percentage of such soaps formed in the intestine during digestion of fat must be very small under normal condition, and hence their solution by the bile is of no great physiological moment. Such solubilities as are quoted above, low though they be, are in any case more than sufficient to account for the absorption of such minimal amounts of calcium or magnesium soaps as may be formed during fat digestion.

4. LECITHIN.—The power which aqueous solutions of bile-salts possess of taking up a large quantity of lecithin into *clear* solution at body temperature is very interesting from the point of view of the re-absorption of the bile-salts, as is also the fact that in presence of lecithin the solvent power is greatly increased for other fatty substances, such as the free fatty acids and soaps, as is shown by the foregoing figures.

Pure lecithin is practically insoluble in water, the addition of as little as 0.1 per cent. causes an opalescence, and further additions give rise, as is well known, to a kind of emulsion. But when lecithin is added to a 5 per cent. solution of bile-salts,* the appearances observed are quite different.

The lecithin dissolves to a clear brown-coloured solution, and the amount taken up is surprising; thus a 5 per cent. solution takes up no less than 7 per cent. of lecithin at a temperature of 37° C. On cooling, part of the lecithin is thrown out of solution as a finely suspended precipitate or emulsion, which glistens with a silky lustre when the test-tube containing it is shaken so as to set the fluid in motion. At ordinary room temperatures of 15° to 20° C., a considerable amount of lecithin, 4 to 5 per cent., is, however, still retained in solution.

The power of lecithin in increasing the solubilities of the fatty acids and soaps, explains in great part why lower solubilities are obtained in experimenting with pure bile-salt solutions, than with bile. The lecithin naturally occurring in bile thus increases the solvent power of that fluid in the intestine for fatty acids and soaps.

5. CHOLESTEARIN.—After the high solubility obtained for lecithin, we were much surprised at the excessively low solubility obtained for cholestearin, and proceeded as above described to make preparations of pure cholestearin from several different sources. The experimental results obtained were however uniform; in all cases it was found that while cholestearin is appreciably more soluble in bile-salt solutions than in water, in which it appears to be absolutely insoluble, yet the degree of solubility is very low. Thus, in several experiments with ox bile, we were unable to dissolve 0.1 per cent. of cholestearin additional, and as far as we could judge most samples of bile are practically saturated with cholestearin. A 5 per cent. solution of bile-salts dissolves about 0.1 per cent. of cholestearin, and the amount is not very appreciably increased by the simultaneous presence of lecithin; at any rate, the amount dissolved by 5 per cent. of bile-salts *plus* 1 per cent. of lecithin does not exceed 0.15 per cent.

This exceedingly low solubility of cholestearin in bile furnishes an interesting experimental explanation of a well-known clinical fact,

* The same results are obtained when lecithin is added to bile; thus a sample of ox bile dissolved 6 per cent. at 36° C. This shows that bile is not nearly saturated with lecithin under normal conditions of its secretion.

viz., that gallstones so often consist of almost pure cholestearin. On account of the low solubility of cholestearin, the bile (the excretory agent for this substance) must, even under normal conditions, be almost saturated with it. Hence anything which either diminishes the amount of bile-salts in circulation or increases the amount of cholestearin in the circulation, such, for example, as increased metabolic changes in the nervous tissues, may cause a supersaturation of the bile with cholestearin, and a deposition of that substance. Such a deposition would occur most commonly in the gall bladder where the supersaturated bile is stored for a time, and where absorption of water and probably of bile-salts also occurs, lowering the solvent power of the contained bile. When precipitation from solution does take place, as is well known under such conditions, the deposition will occur most readily around any nidus of foreign material, such as an epithelial cell.

In such conditions, it is obviously the supersaturation of the bile with cholestearin which is the primary predisposing factor to gallstone formation, and not the presence of the epithelial cell. When a stone is once started, like a crystal already formed in a solution, its surface is a favourable situation for continued deposit, and so the stone continues to increase in size. The ringed appearance of the cross section is probably due to alternations in the rapidity of growth, the bile being more saturated with cholestearin at some periods than at others. Lecithin and the other constituents of the bile, with the exception of the bile pigments, being very soluble are not represented in the composition of gallstones.

CONCLUSIONS.

1. Bile has a dual function as a solvent: (*a*) it acts as a solvent for lecithin and cholestearin, and hence aids in the excretion of those otherwise insoluble bodies by the liver cells, and in their carriage to the intestine; (*b*) it acts as a solvent in the intestine for both free fatty acids and soaps, conferring their entire solubility on the former, and largely increasing the solubility of the latter.

2. These solvent properties of the bile are chiefly due to the bile salts; but in the case of the fatty acids and soaps the amount dissolved is greatly increased by the simultaneous presence of lecithin.

3. These solvent actions of the bile salts explain the utility of the reabsorption of the bile-salts and their circulation through the liver, so that they may be used over and over again as solvent agents. In absorption, the bile salts carry the soaps of fatty acids into the columnar cells; in the liver, they are absorbed by the liver cells, carry the excretory lecithin and cholestearin with them, and are passed into the bile canaliculi holding these substances in solution; in the bile, the lecithin and cholestearin are carried in solution to the intestine; and in the intestine, the soaps and fatty acids are dissolved and rendered capable of

being taken in along with the bile-salts by the columnar cells, while the lecithin and cholestearin which are incapable of absorption are precipitated as the bile-salts are absorbed.

4. Lecithin possesses a high solubility in the bile, and cholestearin a very low solubility. The low solubility of cholestearin furnishes an explanation of the fact that gallstones are composed almost entirely of this substance.

5. The sodium soaps possess only a low solubility in water, the palmitate and stearate being practically insoluble; but the solubility is increased by the presence of bile-salts, and especially in the presence of lecithin; further, the character of the solution is different in the two cases, being less colloidal when in bile-salt solution.

6. Even in bile or bile-salt solution the calcium and magnesium soaps have a low solubility, but of the two the magnesium soaps are the more soluble.

7. These results cast some light on the relative functions of the pancreatic juice and bile in fat digestion and absorption. The enzyme of the pancreatic juice splits up the neutral fats, forming free fatty acids, which are largely converted into soaps by the alkali present; while the bile gives solubility to the fatty acids and soaps so produced. Now it is well known that the fat-absorbing power is impaired but not completely destroyed by the absence of either one secretion, but is practically lost when both secretions are absent. These facts can probably be best explained as follows:—(a) In the absence of the pancreatic ferment, since the bile has no action upon neutral fats, and these are insoluble, only that portion can be absorbed which is free in the fat when ingested, or is set free in the stomach, or by bacterial action in the intestine. Since bacterial action is at a minimum in the small intestine, the fat in great part is not set free until the large intestine is reached, when the bile salts have all been reabsorbed, and hence cannot assist in solution. Accordingly, in the absence of the pancreatic secretion, a large percentage of the fat appears as fatty acids in the fæces. (b) In the absence of the bile, although the fat is decomposed high up in the intestine and converted into fatty acids and soaps, the absorption is slow because the solvent action of the bile is wanting, and hence only a fraction is absorbed, and the remainder passes on chiefly as fatty acid to be thrown out in the fæces. When both pancreatic secretion and bile are absent, in the first place only a small amount is decomposed in the small intestine, and in the second place there is nothing to confer solubility on this small portion, with the result that absorption falls almost to zero.
